UNIVERSITY OF PENNSYLVANIA

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JOHN K. EDWARDS

A DISSERTATION

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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CYTOLOGICAL STUDIES OF TOXICITY IN MERISTEM CELLS OF ROOTS OF ZEA MAYS. I. THE EFFECTS OF THE NEUTRAL SALTS [1]

John K. Edwards

Since the classical researches of Ringer (1884) on the influence of saline media on certain fishes, much work has been done on the biological effects of variations in the salt environment of plants and animals. Investigators in both plant and animal physiology early noted the necessity of a balanced salt medium for the normal functioning of living organisms. Excesses or deficiencies resulted in a retardation of growth and development, or, in some instances, death. Plant physiologists have generally explained the results of salt deficiencies as starvation. While this explanation may be correct for long-time studies where certain elements are deficient but not entirely absent, the results of short-time studies are probably best explained as due to the toxicity of the remaining elements (True, 1930; Bamford, 1931).

The studies of True and his associates (1896, 1900, 1930) on the toxicity of single-salt solutions indicate four general classes of inorganic plant poisons: the acids and acid salts, salts of the alkali metals and earths, compounds of the heavy metals, and salts with toxic anions. Solutions of the acids and acid salts are relatively toxic, depending on the hydrogen-ion concentration. The neutral salts of the alkali metals and earths, weakly toxic in most cases, vary in their toxicity as the cation is changed. With the heavy metals, all extremely toxic, the metallic ion is the cause of injury. The common anions most toxic to plant protoplasm are those of the fluorides, bromides, iodides, and cyanides.

The neutral salts of potassium, sodium, magnesium, and calcium are of especial interest to the physiologist. All form a part of the normal environment of plants and animals. Sodium is apparently the only one of the group not necessary in plant nutrition; yet its close chemical affinity to potassium and its universal presence in nature have caused it to be included in most studies of this series. The majority of papers reporting the various biological effects caused by these salts express results largely in terms of gross morphology or as visible physiological reactions in living material. Comparatively little has been done on the cytological reactions of the cells and tissues. The present investigation was made in an effort to determine the cytological changes in the root-tip cells of *Zea Mays* following toxic treatments with the chlorides of potassium, sodium, magnesium, and calcium.

Various plant parts have been used in studying the effects of the neutral salts on protoplasm. Addoms (1927) immersed root hairs of wheat in 0.1 N single-salt solutions, and observed the effect on the protoplasm under dark-field illumination. The salts of potassium, sodium, calcium, magnesium, zinc, and aluminium all caused coagulation. Kerr (1933) noted that 80 per cent of the root hairs of *Limnobium* burst after a few minutes in 0.02 M NaCl, the remaining 20 per cent dying within 30 minutes. Similar results with the salts of potassium have been reported by Cholodnyj (1923). Kahho (1921), in a study of the effects of neutral salts on cells of *Tradescantia* and red cabbage leaves, found the following order for the chlorides, beginning the series with the most toxic: $K > NH_4 > Na > Sr > Mg > Ba > Ca$. The inability of the cells to plasmolyze in a strong sugar solution after the salt treatment was used as an indication of the coagulation and death of the protoplasm. Iljin (1935), in a similar study, investigated the killing of plant cells by immersing epidermal tissue in various concentrations of KCl, NaCl, and $CaCl_2$. In the non-toxic concentrations the cytoplasm and nucleus were without visible structure, or, at the most, finely

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granular. Stronger concentrations caused a granulation and coagulation of the cell parts. The changes in the cytoplasm were gradual, beginning in the region of the nucleus and spreading throughout the cell. The tonoplast was the last part to die, retaining its semipermeable character in strong solutions long after the other parts were dead. Seifriz (1923) found that the salts of potassium, sodium, and calcium in 0.128 M concentrations stopped protoplasmic streaming in *Elodea* leaf cells after a few hours.

Injection experiments where the salt solutions are placed in direct contact with the cytoplasm have yielded interesting results. Skeen (1930) injected a $CaCl_2$ solution (containing 20 p. p. m. Ca) into *Trianea* (*Limnobium*) root hairs and noted that cyclosis stopped in five minutes, with death resulting from coagulation in 10–15 minutes. Kerr (1933), working with root hairs of *Limnobium*, found an irreversible coagulation of the cytoplasm with $CaCl_2$ stronger than 0.04 M, whereas the coagulation produced by 0.1 M $MgCl_2$ was reversible. KCl and NaCl in 1 M concentrations mixed freely with the protoplasm and stopped cyclosis for only five to ten minutes.

Most cytological studies of cells showing chemical injury have been made on material grown in unbalanced nutrient solutions. Reed (1907) found that mitosis continued in root tips of *Zea Mays* in the absence of calcium, but new cell walls were not formed. Sorokin and Sommer (1929) made cytological studies on the roots of *Pisum sativum* grown in nutrient solutions lacking calcium. They reported a material decrease in the amount of cytoplasm, irregular mitotic as well as amitotic cell division, and a failure of the embryonic cells to mature. In solutions lacking both calcium and magnesium, the tissues underwent a slower degeneration, but otherwise were much the same as found when calcium alone was lacking. This retardation of injury is explained by the absence of microorganisms from the magnesium-free solutions. Bamford (1931) observed the root tips of wheat and corn grown in calcium-deficient nutrients. Where calcium was entirely absent, growth ceased almost immediately after the roots were placed in the medium. Cell injury progressed from the periphery of the root inward and was characterized by a gradual "erosion" of the cytoplasm, followed by degeneration and disappearance of the nucleus. As wheat roots were normal after four days in distilled water, the pronounced injury at the end of two days in the calcium-free solution was attributed to the other constituents of the nutrient, especially magnesium. Lutman (1934) grew various higher plants in nutrient solutions in which either nitrogen, phosphorus, potassium, magnesium, or calcium was lacking. Root-tip cells of secondary roots grown in these solutions showed a premature vacuolization, interpreted by Lutman as indicating maturity and senescence. The nucleus is reported smaller in relation to cell size in the solutions deficient in nitrogen, phosphorus or magnesium.

Yamaha (1927) has given a detailed account of the effects of many different chemicals on the root-tip cells of *Vicia faba*. The roots were placed in various concentrations of the salts for a period of two hours, followed by washing in running water and fixation in Flemming's fixative. With the chlorides of potassium, sodium, and calcium in the higher concentrations (2 M to 0.02 M), he reports coagulation of the cytoplasm and certain mitotic anomalies.

Materials and methods.—Young seedlings of *Zea Mays* L. (Golden Beauty) furnished the plant material for the experimental work. When germinated in moist sphagnum for four days at 19–22°C., about 40–50 per cent of the seedlings have primary roots 5–6 cm. long. As profuse laterals are not developed until the sixth or seventh day, seedlings of such age are very useful in short-time growth studies. The experimental set-up was the same as that described by Beall (1936), consisting of 500 cc. pyrex beakers with perforated pyrex Petri dishes supporting the seedlings. Such a set-up insures immersion of the entire radicle with the exception of the upper 10 mm. The toxic solutions were prepared from 1 M stock solutions of Merck's reagent $CaCl_2$, $MgCl_2$, and KCl, and Merck's c.p. NaCl. During the time of exposure to the toxic solutions, the plants were kept in an insulated dark room, with air and solution temperatures varying from 19–22°C.

Preliminary growth studies were made with ten concentrations of each salt, ranging from 0.1 M to 0.01 M for KCl, NaCl, and $MgCl_2$, and from 0.2 M to 0.01 M for $CaCl_2$. The failure of the roots to elongate in distilled water after 24 hours in the salt solutions was used as a criterion of death, while additional growth in distilled water indicated survival and recovery. On the basis of the preliminary studies, the toxic treatment of material for cytological study was limited to four concentrations of each salt, ranging from strongly toxic through less toxic concentrations to solutions where growth was only slightly less than normal. The $CaCl_2$ series was 0.2 M, 0.1 M, 0.033 M, and 0.01 M. For the potassium, sodium, and magnesium salts, the concentrations used were 0.1 M, 0.033 M, 0.02 M, and 0.01 M. At the end of the toxic treatments lasting 24 hours, the root tips were fixed in each of four cytological fixatives. The fixing fluids used were a chromic sulphate-formaldehyde mixture (Zirkle, 1932a), Zirkle's (1934) modification of Erliki's solution, Bouin's, and Nawaschin's fluid. The fixation time was 48 hours. Dehydration in the butyl alcohol series was followed by embedding in paraffin. The sections were cut eight micra in thickness, and stained with iron-alum haematoxylin. No counter stain was used.

Cells of the normal root tips of Zea Mays.—The root tip of *Zea Mays* has the embryonic tissue organization typical of the majority of monocotyledons. A distinct calyptrogen covers a single layer of initials which immediately give rise to the plerome cylinder, periblem, and dermatogen of the root. The cells near the tip of seminal roots tend to be isodiametric,

measuring about 10–15 micra in size. The nuclei are comparatively small and spherical, normally occupying the center of the cell. Dividing cells are abundant at all times, but are slightly more numerous during the day (Karsten, 1915). Early prophase stages are plentiful and show a well defined spireme. The chromosomes are long and numerous and tend to be fixed in clumps at metaphase and telophase. Although the cells of the initial region are generally reported to be non-vacuolate and rich in cytoplasm, the work of Zirkle (1932a) shows the presence of numerous spherical vacuoles, varying from four to eight micra in diameter in the calyptrogen and plerome, and somewhat smaller in the other tissues. Although careful work on the nucleo-cytoplasmic ratio has not been reported, it is quite likely that cells of the primary meristems resemble the cambium cells observed by Bailey (1930), where the more rapidly dividing cells, instead of being richer in cytoplasm, are the more vacuolate.

Fixation in the normal meristem cells of Zea Mays.—From his extensive work on fixation, Zirkle (1928, 1932a, 1932b, 1934) recognizes two definite types of fixation image, the so-called "acid" and "basic" images. The acid images are those produced by fixing solutions containing acetic acid, and by chromic acid and the bichromates on the acid side of pH 4.8–5.0. The basic images are set by formaldehyde and by certain metallic salts on the basic side of pH 4.8–5.0. The fluids producing the acid image are the best preservatives of resting and dividing chromatin, nucleolar material, and spindle fibers. The cytoplasm, however, is fixed as a reticulum, or as fairly coarse threads, and is referred to as "spongioplasm." All cytoplasmic detail is destroyed and the resulting image, including the vacuoles, is wholly artificial. The basic images are characterized by a fixation of the cytoplasm as "hyaloplasm"—that is, without any visible structure. Although the tonoplast is dissolved, the vacuoles are preserved in their true form. Mitochondria, the nucleolus, nuclear lymph, and dividing chromatin are fixed, while resting chromatin is dissolved.

A critical study of the staining properties of the root-tip cells following various acid and basic fixations has been made by Carlson (1936). He finds that the basic fixations of chromic sulphate-formaldehyde and modified Erliki's solutions give similar images and stain well with iron-alum haematoxylin. He also finds that the acid fixations of Bouin's and Nawaschin's fluids are much alike when followed by the same staining technique.

Of the fixatives used in this study, two produce the acid image and two the basic image. The chromic sulphate-formaldehyde mixture preserves the cytoplasm much as it is seen in the living condition (Zirkle, 1932a), and, except where otherwise indicated, conclusions regarding the effects of the toxic solutions on cytoplasmic structure and vacuoles are drawn from a study of material fixed in this medium. The modified Erliki's fixative, although erratic at times, fixes mitochondria better than the chromic sulphate-formaldehyde solution, and is used to determine their presence. Injury to resting and dividing nuclei is determined from material fixed in Bouin's and Nawaschin's fixatives.

Results of cytological studies.—*The cytoplasm following basic fixation.*—The cytoplasm of root-tip cells is markedly affected by strong concentrations (0.1 M) of KCl and NaCl, but tends to assume a normal appearance in the less toxic solutions. Basic fixation following death of the roots in 0.1 M solutions shows a tendency for the cytoplasm to pull away from the cell walls and clump about the nucleus (fig. 2, 3). The resulting vacuoles are large and distorted, and probably represent a fusion of the small spherical vacuoles of the normal cell. Potassium, in the case of the 0.1 M solutions, produces a coarsely granular cytoplasm, while sodium of the same strength causes little change from the normal appearance of the cytoplasm. The 0.033 M treatments produce an image similar to that of the 0.1 M solutions, except that true vacuoles, normal in size and appearance, may be seen in quite a few cells. With the 0.02 M concentrations, a great number of large vacuoles persist in an otherwise normal cytoplasm. All cells appear normal after 24 hours in the 0.01 M solutions. Although the sections were not destained especially for observation of mitochondria, the latter are seen in some cells of the root tips following all sodium and potassium treatments.

$MgCl_2$ is very similar to KCl and NaCl in its action on the cytoplasm (fig. 4). The 0.1 M solutions cause a clumping around the nucleus, leaving the fused vacuoles as large clear spaces. In most cases the cytoplasm is fixed as a homogeneous mass (hyaloplasm), but in some cells small dark-staining globules are present. As normal nuclei are often present in the same cells, no postulations are made regarding the origin of this chromatin-like material. The 0.033 M and 0.02 M concentrations cause much the same type of fused vacuoles, comparatively few being normal. Roots from the 0.01 M treatment show cells with normal cytoplasm and vacuoles. Mitochondria were observed in all but the 0.1 M series.

Although not so drastic in its killing action as 0.1 M KCl, the 0.2 M $CaCl_2$ affects the cytoplasm in a similar manner. The cytoplasm is seen to contain small evenly distributed granules, probably formed by the salt solution prior to fixation. The vacuoles tend to be large and distorted, and apparently represent a coalescence of the smaller normal vacuoles. The non-killing concentrations produce a less granular cytoplasm and a higher percentage of normal vacuoles as the dilutions become progressively greater, until a normal basic image is secured following the 0.01 M treatment. Mitochondria are present in all cases.

Although the antagonistic effects of calcium on the injurious action of other salts were not thoroughly investigated, the cytoplasm and vacuoles appear nor-

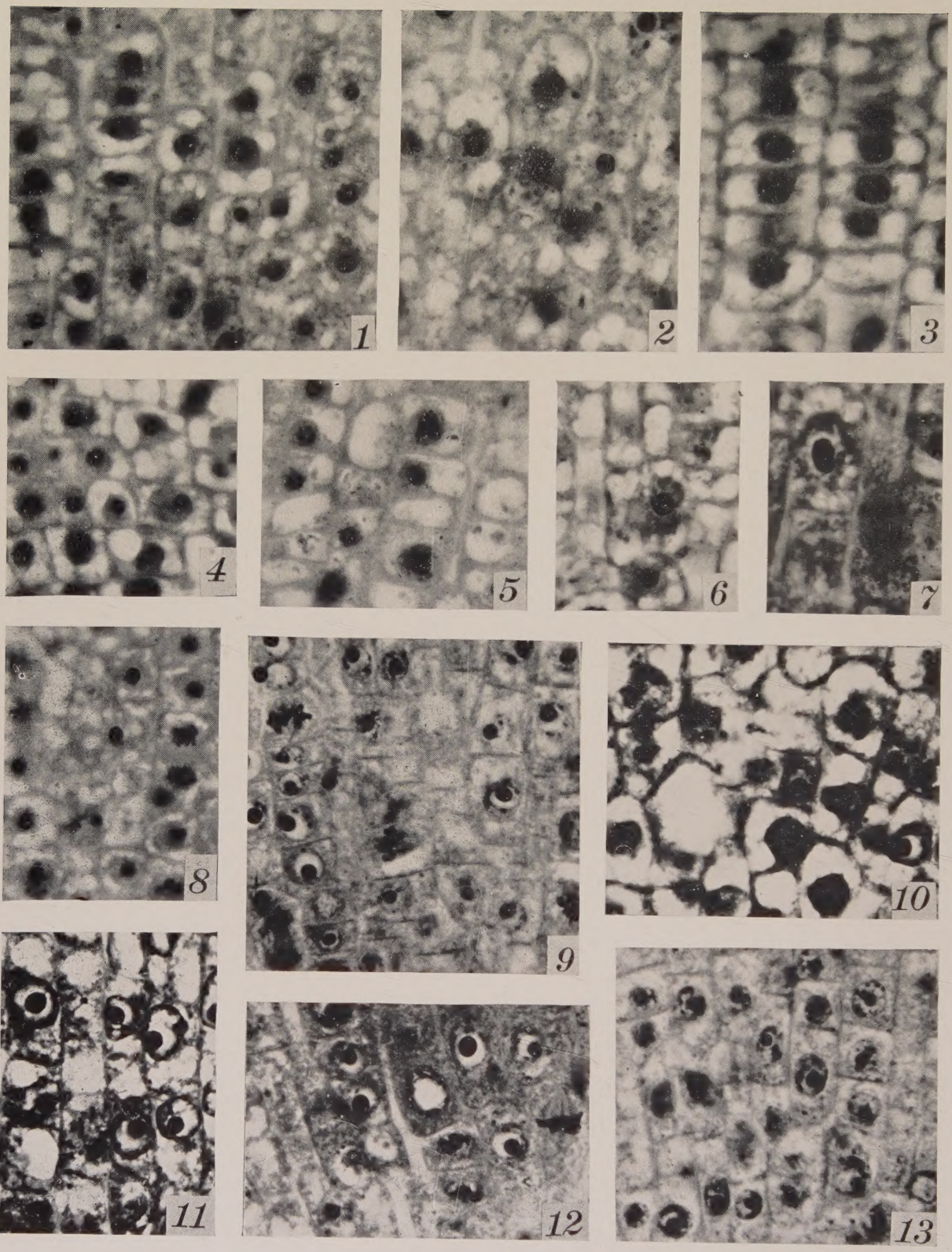

Fig. 1–13. All figures represent undifferentiated cells from the region immediately behind the initials. The magnification is 838 diameters. Fig. 1–8 show the basic fixation images of chromic sulphate-formaldehyde following the various indicated treatments, while fig. 9–13 show the acid images of Bouin's fixative.—Fig. 1. Cells from normal root tip grown in distilled water.—Fig. 2. 0.1 M KCl.—Fig. 3. 0.1 M NaCl.—Fig. 4. 0.033 M $MgCl_2$.—Fig. 5. 0.2 M $CaCl_2$.—Fig. 6. 0.033 M KCl.—Fig. 7. 0.02 M KCl.—Fig. 8. 0.033 M $CaCl_2$.—Fig. 9. Cells from normal root tip grown in distilled water and fixed in Bouin's.—Fig. 10. 0.1 M KCl.—Fig. 11. 0.1 M NaCl.—Fig. 12. 0.033 M KCl. Fig. 13. 0.2 M $CaCl_2$.

TABLE 1. *Summary of the effects of the neutral salts on various parts of the cell.*[a]

	KCl				NaCl				$MgCl_2$				$CaCl_2$			
Cell parts	M/10	M/30	M/50	M/100	M/10	M/30	M/50	M/100	M/10	M/30	M/50	M/100	M/5	M/10	M/30	M/100
Cytoplasm	CG	CG	N	N	CS	CS	N	N	CS	CS	CS	N	CG	CG	CG	N
Vacuoles	D	D	DN	N	D	D	DN	N	D	D	DN	N	D	DN	DN	N
Chromatin	CG	GN	N	N	CG	GN	N	N	CG	GN	N	N	CG	CG	N	N
Prophases	F	N	N	N	F	N	N	N	F	N	N	N	F	N	N	N
Metaphases	F	N	N	N	A	N	F	N	A	N	N	N	A	N	N	N
Anaphases	F	N	N	N	A	N	F	N	A	N	N	N	A	N	N	N
Telophases	F	N	N	N	A	N	F	N	A	N	N	N	A	N	N	N
Spindles	A	N	N	N	A	N	F	N	A	N	N	N	A	N	N	N
Cell plates	N	N	N	N	N	N	N	N	A	N	N	N	A	N	N	N
Nucleoli	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mitochondria	P	P	P	P	P	P	P	P	A	P	P	P	P	P	P	P

[a] N, normal; D, distorted; C, clumped; G, granular; A, absent; P, present; F, few; S, smooth.

mal in cells of roots grown in a solution containing 0.2 M KCl and 0.01 M $CaCl_2$.

The cytoplasm following acid fixation.—Root tips grown in distilled water for 24 hours and fixed in Bouin's fluid show the typical acid image, with a granular, evenly distributed cytoplasm (fig. 9). The 0.1 M solutions of KCl, NaCl, and $MgCl_2$, however, produce images very similar to those described by Sorokin and Sommer (1929) and by Bamford (1931) for calcium-starved roots. A majority of the cells are practically devoid of cytoplasm, while the remainder show large clear spaces, not typical of true vacuoles (fig. 10, 11). In striking contrast to this condition is the image obtained following treatment with 0.2 M $CaCl_2$ (fig. 13). Here the cytoplasm is granular and evenly distributed throughout the cells, much as the normal condition following acid fixation. The weaker concentrations of the various salts do not noticeably affect the acid fixation of cytoplasm.

Resting and dividing nuclei following acid fixation.—The acid fixatives show well preserved nuclei in the cells of roots grown in distilled water. The nuclear lymph is dissolved, leaving a clear halo about the well-fixed nucleoli. The resting chromatin is fixed as granules of various sizes, generally in the peripheral region of the nucleus. Division figures are abundant and show normal spindle fibers and cell plates.

Only the more concentrated salt solutions cause noticeable injury to the resting nucleus (fig. 10, 11). Following exposure to these, some nuclei are angular or elongated, while others retain their normal spherical shape. All salts react alike in causing a clumping of the chromatin, either as large granules or as dark-staining amorphous masses around the periphery of the nucleus. Following immersion in 0.1 M KCl this chromatic material appears to occupy most of the space left by the dissolved nuclear lymph, leaving a narrow, irregular halo around the nucleolus. Except for a slightly more granular chromatin, the resting nuclei are practically normal after 24 hours in the 0.033 M solutions (fig. 12), while the 0.02 M and 0.01 M concentrations produce no noticeable changes. The nucleoli appear normal in all cases, retaining their staining qualities and spherical shape.

The effects of the salt solutions on dividing cells point to two types of toxic action. Potassium and sodium kill root tips in 0.02 M concentrations, yet allow normal mitosis in 0.033 M solutions. Calcium and magnesium, however, show a total absence of division figures in all roots subjected to killing concentrations of the salts. Of the two alkali metal salts, KCl seems to have the least effect on mitosis. As a study of table 1 indicates, even the 0.1 M KCl series shows a few mitotic figures, and all other tested concentrations allow normal cell divisions. Sodium reacts in a slightly different manner, as mitotic figures are entirely absent from the cells of roots subjected to the 0.1 M concentrations, while the 0.02 M series shows very few dividing cells. The effect of the 0.033 M treatment, however, is similar to that of potassium in that normal mitosis takes place. This scarcity of dividing cells following exposure to the 0.02 M NaCl was consistent in all observed material, consisting of four root tips fixed in Bouin's fluid, four in Nawaschin's, two in the chromic sulphate-formaldehyde mixture, and two in modified Erliki's. With the exception of the 0.02 M NaCl, the three less-concentrated solutions of each salt produce an image very similar to that shown for 0.033 M KCl (fig. 12). The tendency for metaphase chromosomes to clump is typical for Bouin's fixative.

Resting and dividing nuclei following basic fixation.—While the basic fixation image is not so useful for nuclear study, all parts except the resting chromatin are preserved, making additional observations on the effects of the salt treatments on nuclei possible. The nucleolus, aside from deviations from the spherical shape, is practically the same in all cases. None of the salts destroyed the nuclear lymph, and the halo around the nucleolus so typical of acid fixation is missing. Division figures, while not so abundant as with the acid fixations, were observed in some cases following the weaker salt treatments. However, any scarcity or absence of dividing cells following basic

fixation may often be due to slow penetration of the fixative or to its dilution by substances already present in the cells.

Roots grown in 0.2 M KCl antagonized with 0.01 M $CaCl_2$ showed an abundance of mitotic figures following fixation in chromic sulphate-formaldehyde.

Discussion.—The results reported in the present paper, while not comparable to the cellular degeneration in roots after many days in calcium-free nutrients, may properly be compared to the results reported at the end of one or two days in such media.

Many workers have reported that calcium in small amounts antagonizes all ordinary constituents of plant-nutrient solutions, which taken alone or in mixed solutions are toxic to plant protoplasm. Since root-tip cells of *Zea Mays* are normal after one day, and wheat after four days in distilled water (Bamford, 1931), it is evident that the seed furnishes sufficient calcium to supply the newly formed protoplasm and, at the same time, prevent marked injury by any other salts made soluble by the processes of germination and growth. With young seedlings it is only when other salts are introduced into the external medium that additional calcium from an outside source becomes necessary for the preservation of the cells in a normal living condition. Consequently, any explanation of injury to cells of seedlings grown in calcium-deficient nutrients, when control plants grown in distilled water are still normal, should take into consideration the toxic effects of the remaining salts. Some idea of the potential toxicity of a nutrient solution lacking calcium may be gained by calculating the total potassium and magnesium in the solutions used by Sorokin and Sommer (1929). Their solution without calcium contained a total of 0.57 gm. of potassium and 0.049 gm. of magnesium per liter, as well as traces of sodium, manganese, aluminium, copper, iodine, and fluorine, all harmful to plants in the absence of calcium. By way of comparison, the amount of potassium in the weakest killing concentration of KCl is 0.78 gm. per liter.

The cytological reactions produced in root-tips of *Zea Mays* by single-salt solutions of KCl, NaCl, $MgCl_2$, and $CaCl_2$ have been described. A comparison of the effects of the salts on the cytoplasm, as shown by acid and basic fixations, is of interest, especially since all previous workers have drawn their conclusions from a study of material fixed in acid fixatives. Sorokin and Sommer (1929) report a decrease in the amount of cytoplasm and the appearance of large vacuoles as one of the first visible effects of calcium starvation on roots of *Pisum sativum*. In a similar study with corn and wheat, Bamford (1931) also records an early disappearance of the cytoplasm. He emphasizes, however, the toxicity of the remaining elements of the culture solution, while Sorokin and Sommer explain all injury strictly in terms of calcium absence. They conclude that calcium is necessary to the protoplasm, either as a chemical constituent or because of its physical effects on the colloidal system of the cell. Lutman (1934) also reports less cytoplasm and increased vacuolization when plants are starved for certain elements, especially calcium, magnesium, and potassium, and concludes that such cells have reached early maturity and senescence due to starvation.

Although basic fixation shows that the cytoplasm is definitely injured at the end of one day in 0.1 M solutions of KCl, NaCl, and $MgCl_2$, the injury is evident only as clumping and increased granulation. The many small vacuoles typical of normal root-tip cells are fused into a few larger ones. No treatment causes a definite loss of cytoplasm. On the other hand, when roots from the same salt treatments are fixed in an acid fixative, the results are strikingly different. The cytoplasm, fixed in the normal cell as a non-vacuolate spongioplasm, reacts with the salts in such a way that it is either dissolved or is badly shrunken by the fixing solution. The presence of $CaCl_2$, however, even as a 0.2 M single-salt solution, predisposes the cytoplasm to the usual acid fixation with the acid fixatives. It will also be recalled that 0.01 M $CaCl_2$ is sufficient to prevent injury to the cytoplasm, as is revealed by basic fixation. It would seem, then, that injury to the cytoplasm is best interpreted from material fixed in basic fixatives. Just as the long-accepted idea that meristem cells are non-vacuolate has been based on observations of the artificial cytoplasm of the acid fixation image, so the apparent early loss of cytoplasm from cells subjected to toxic treatments, or calcium starvation, is based on an artifact of acid fixation.

Most investigators who have observed the effects of the neutral salts on plant cells, whether as single-salt solutions or as calcium-free nutrients, agree that the nucleus is the last part of the cell to show signs of injury. Reed (1907) studied the cells of several types of plants after calcium starvation and found no modifications in the form of the nucleus, or in the amount, form, or arrangement of the chromosomes. He did, however, record a marked absence of cell plates in dividing cells of *Spirogyra* and *Zea Mays*. Sorokin and Sommer (1929) reported a disturbance of the mitotic axes in dividing cells of *Pisum sativum* grown from one to two days in a calcium-free nutrient, while Bamford (1931) found that the nuclei remain normal in wheat roots during the first few days in solutions without calcium, nuclear degeneration being one of the late stages of cellular injury. With the exception of slight differences in the size of the nuclei, Lutman (1934) found no fundamental variations from the normal condition when unbalanced nutrients were used. Yamaha (1927) subjected roots of *Vicia faba* to salt solutions of various concentrations (2 M to 0.000002 M) for a two-hour period. The stronger solutons kill all cells and leave no definite structure; the weaker solutions, while not killing in their action, are reported to cause various types of mitotic anomalies. Following 0.02 M KCl treatment, there is a thickening and shortening of the chromosomes, a degeneration of the spindle fibers, and an absence of cell plates in some cells. $CaCl_2$

and NaCl cause reactions similar to those produced by the KCl. Nothing resembling these mitotic aberrations is found in roots of *Zea Mays* after 24 hours in the salt solutions. The stronger concentrations of the salts tend to prevent cell division, while the weaker solutions allow typical mitosis. If the dividing cells of *Zea Mays* are as definitely abnormal at the end of two hours in the salt solutions as Yamaha reports for *Vicia faba*, they are able to adapt themselves to such an extent that only normal mitosis is taking place at the end of 24 hours. The granulation and clumping of resting chromatin reported in this investigation is upheld by Iljin's (1935) observations of the coagulation and clumping of the nucleus in living material when subjected to toxic concentrations of the neutral salts. Longer exposure of root tips to the toxic solutions would undoubtedly bring about a degeneration of the nucleus, with a possibility of pseudomitosis or amitosis before the cell was completely dead.

SUMMARY

The toxic effects of the chlorides of potassium, sodium, magnesium, and calcium upon cells of root tips of *Zea Mays L.* were investigated. The roots were exposed to various concentrations of the salt solutions for 24 hours, followed by fixation for 48 hours in acid and basic fixatives.

The roots were dead at the end of 24 hours in 0.02 M KCl and NaCl, 0.1 M $MgCl_2$, and 0.2 M $CaCl_2$, as shown by their inability to continue growth in distilled water.

Basic fixation of root tips after the toxic treatments showed a clumping of the cytoplasm about the nucleus. The resulting vacuoles were large and represented a fusion of the smaller vacuoles of the normal cell. A definite decrease in the amount of cytoplasm was not observed.

The cytoplasm in cells of root tips exposed to 0.1 M concentrations of KCl, NaCl, and $MgCl_2$ was either dissolved or badly shrunken by the acid fixatives. The more dilute solutions of these salts, and $CaCl_2$ in all tested concentrations, had little effect on the acid fixation image.

Except for a clumping of the chromatin by strong concentrations of the salts, there were no fundamental changes in the resting nucleus.

$MgCl_2$ and $CaCl_2$ in killing concentrations prevented mitosis, while KCl and NaCl were toxic in 0.02 M concentrations but allowed normal mitosis in 0.033 M solutions. Aberrant mitotic figures were not observed.

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